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Title Derwent

New dihydroliponamide dehydrogenase from dogfish, useful as diaphorase for converting substrates to dye, in analysis, and for producing enantiomerically pure lipoic acid

Abstract Derwent

Novelty: Protein (I) with the activity of a dihydroliponamide dehydrogenase is a homodimer of relative molecular weight 100-150 kDa (gel filtration) and relative molecular weight of the subunits 50 kDa (sodium dodecylsulfate polyacrylamide gel electrophoresis), is new.

Description: Protein (I) with the activity of a dihydroliponamide dehydrogenase is a homodimer of relative molecular weight 100-150 kDa (gel filtration) and relative molecular weight of the subunits 50 kDa (sodium dodecylsulfate polyacrylamide gel electrophoresis), is new. (I) is prepared by:(a) producing a crude extract of muscle tissue from the dogfish *Scyliorhinus canicula*;(b) gel filtration through a Superdex (RTM) column; and(c) anion-exchange chromatography. An INDEPENDENT CLAIM is also included for preparation of (I).

Activity: Cytostatic; anti-HIV (human immunodeficiency virus); antidiabetic; hepatotropic; antiarteriosclerotic. No biological data is given.

Mechanism of Action: None given.

Use: (I), and related enzymes from other fish of the family Scyliorhinidae, are used (claimed) as diaphorase, particularly for oxidative or reductive conversion of a substrate to a dye, i.e. for (In)direct detection (in the visible spectrum) of substances such as amino acids, proteins, nucleic acids, and co-factors, in coupled enzyme substrate tests (claimed). Other uses for (I) are in synthesis of enantiomerically pure (or enriched) lipoic acid, particularly in the (R) conformation, useful as e.g. radical scavenger, antioxidant (regeneration of Vitamin C and potentiation of Vitamin E activity), protection of membranes against lipid peroxidation, or of nucleic acids or proteins, e.g. for prevention and treatment of cancer, acquired immune deficiency syndrome, diabetes, liver cirrhosis, polyneuritis, arteriosclerosis, lactate acidosis and heavy metal poisoning.

Advantage: (I) has a low temperature maximum and high specificity at low temperature, so catalyzes reactions rapidly at room temperature, eliminating the need for, and expense of, thermostating. It has relatively good temperature stability (allowing long-term storage at ambient temperature) and its enzymatic activity can be reversed by altering the temperature (functioning as temperature switch), i.e. increasing the temperature to 70 degrees C suppresses activity but activity is restored on cooling.

Wider Disclosure: Antibodies (Ab), or their fragments, specific for (I) or its fragments, are disclosed as new.

Example: Parietal muscle from *Scyliorhinus canicula* was frozen in liquid nitrogen, crushed to powder and this taken up in 10 mM potassium phosphate (pH 7.5). The mixture was incubated at 80 degrees C for 10 minutes, with stirring, then centrifuged at 16000 g and 4 degrees C for 10 minutes. The supernatant was subjected to gel filtration on Superdex (RTM) 200, using the specified buffer, and active fractions chromatographed on a column of Mono Q (RTM) HR 5/5, eluting with a linear 0-1 M gradient of sodium chloride in same buffer. The active fractions were then ultrafiltered to recover a protein with dihydroliponamide dehydrogenase activity.

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- No drawing available -